

# Toxic Potential of the Plasticizer Di(2-ethylhexyl) Phthalate in the Context of Its Disposition and Metabolism in Primates and Man

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Although human toxicity from exposure to the plasticizer di(2-ethylhexyl) phthalate (DEHP) is unknown, reports of animal toxicity from DEHP have stimulated extensive toxicological studies. In the absence of direct toxicity data, information on the disposition and metabolism of DEHP in primates and man may enhance our assessment of the toxic potential of DEHP in man. Studies of DEHP disposition and metabolism in the African Green monkey and man show that the compound is rapidly and extensively metabolized. It is excreted largely in the urine (> 90%) as conjugated (glucuronide) oxidation products of mono(2-ethylhexyl) phthalate; excretion in feces accounts for the other 10% of the administered DEHP. Plasma disappearance of parenterally administered DEHP is equally rapid so that by 24 hr following DEHP administration, plasma DEHP concentrations are virtually undetectable, while greater than 70% of the dose has been excreted in urine and stool. The transience of DEHP in primates and the extent to which it is metabolized and conjugated may play a role in the observed lack of toxicity.

## Introduction

Interest in the toxic potential of the principal plasticizer contaminant of blood storage, di(2-ethylhexyl) phthalate (DEHP), was kindled by reports of DEHP residues in tissues of patients receiving stored blood products (1). However, discovery of DEHP in tissues of humans who had not received transfusions (2), serious disagreement between research laboratories on results of assay determinations of DEHP in identical aliquots of DEHP-spiked tissue samples (3), and frequent assay contamination with ubiquitous environmental DEHP (4), all have made reports of DEHP detection in human tissues difficult to interpret.

Potential DEHP toxicity has been inferred from numerous animal studies. However, many studies of the toxicity of DEHP in animals have entailed

nonintravenous routes of administration, atypical dosage formulations, and excessive doses which do not imitate the exposure pattern in man (5-7). Correspondingly, the relevance of such studies to human parenteral exposure at relatively low doses of protein-bound DEHP in stored blood products is not amenable to rigorous scientific scrutiny at present. Two exceptions to this criticism bear mention. Garvin et al. (8) reported that no acute or cumulative toxicity could be demonstrated in rats infused twice weekly for 9 weeks with DEHP-rich stored homologous plasma. Each infusion resulted in a DEHP dosage which was equivalent to that which a human adult would receive from a 12-unit whole blood transfusion. On the other hand, Jacobson and co-workers (9) attributed hepatotoxicity to DEHP in rhesus monkeys given weekly autologous infusions of DEHP-rich platelet concentrates for 1 year. The DEHP infused each week was roughly equivalent to that a human would receive from a 2-unit whole blood transfusion. Whether these investigators correctly interpreted their results as DEHP toxicity is uncertain, since the exposure levels of mono(2-ethylhexyl) phthalate (MEHP)

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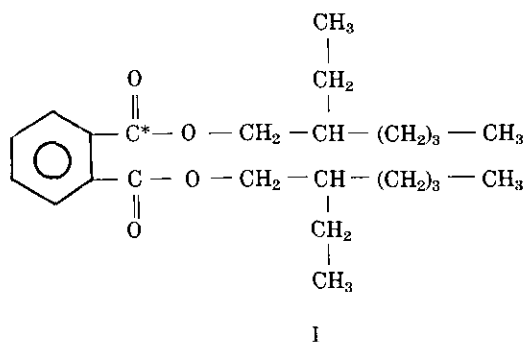
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were not reported (see discussion on the significance of MEHP below). The importance of paying careful attention to the details of comparable dosage, the physical state of the dosing formulation and its composition in the planning and interpretation of DEHP toxicity studies has been emphasized (10).

Although human toxicity to DEHP through blood transfusions has not been documented, there remains a concern for the potential toxicity of this ubiquitous compound. In the absence of direct clinical toxicity data, the potential for various effects may be evaluated from knowledge of tissue distribution, the chemical nature of DEHP metabolites and conjugates, the organs involved in DEHP metabolism and elimination, and the time course of these phenomena. It is in this context that we review current knowledge of the disposition and metabolism of DEHP in primates and man.

## Structure and Leaching of DEHP

The chemical structure of DEHP is shown as I. DEHP consists of a benzene ring to which are attached two carboxyl groups. Each of the latter is coupled to a 2-ethylhexanol side chain by an ester bond. The asterisk on the carbonyl carbon indicates the position of a  $^{14}\text{C}$  label which was used in our studies of the disposition of DEHP in the primate African Green monkey (11-13). Either carbonyl carbon may be the site at which plasma and tissue esterases in primates act to cleave one of the 2-ethylhexanol side chains to produce MEHP.



DEHP is a clear, oily liquid which is highly fat-soluble and poorly water-soluble. In poly(vinyl chloride) (PVC) blood storage containers, the plasticizer resides in the PVC matrix as a semisolid and readily migrates from the plastic into plasma during storage. DEHP accumulates in plasma during  $4^{\circ}\text{C}$  liquid whole blood storage at a rate of approximately 1 mg/unit/day and 6 mg/unit of platelet concentrate/day during storage at room temperature (14).

Studies performed in our laboratory and elsewhere (14-16) indicate that plasma DEHP undergoes hydrolysis to MEHP during storage, presumably under the influence of plasma nonspecific lipase. This reaction results in accumulation of MEHP in whole blood (0.8-2.9 mg/unit after 21 days of storage), as well as in platelet concentrates (0.82 mg/platelet pack after 50 hr of storage). Interest in MEHP stems from evidence indicating that large oral doses of MEHP are hepatotoxic in rodents (17).

## African Green Monkey Studies

We initiated our investigation of the disposition of DEHP in primates by studying the distribution, elimination, and metabolism of this compound in the African Green monkey (11-13, 18). In order to

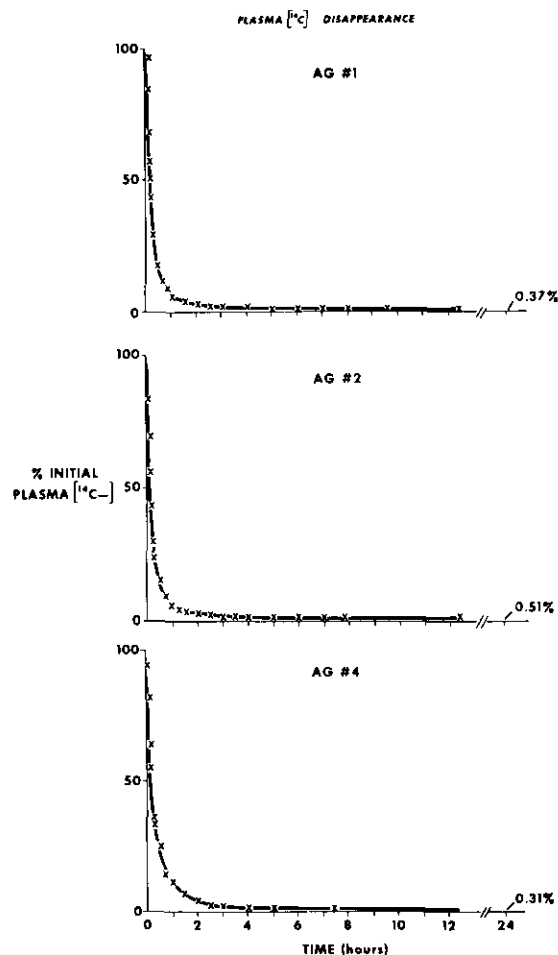


FIGURE 1. Plasma  $^{14}\text{C}$  concentrations in three African Green monkeys (AG 1, 2, 4) following bolus injection of  $^{14}\text{C}$ -DEHP leached into autologous plasma. The solid line running through the data (x) was generated from a computer fit of the data (13).

simulate closely the manner in which man is exposed to DEHP when receiving blood products, we procured a strip of PVC plastic (Fenwal Laboratories, Morton Grove, Ill.) which was impregnated with  $^{14}\text{C}$ -DEHP. This sheet exhibited physical characteristics which were identical to that of Fenwal's PL-146 blood bag plastic. A 20-ml aliquot of plasma was taken from each of three African Green monkeys; PVC strips containing  $^{14}\text{C}$ -DEHP were immersed in these plasmas and stored at  $4^\circ\text{C}$  for up to 5 months. At the time of reinfusion, these plasmas each contained around 3 mg of  $^{14}\text{C}$ -DEHP, which was equivalent to that in a two-unit infusion of 21-day-old bank blood in a human. Thin-layer chromatography (TLC) and gas chromatography mass-spectrometry (GC-MS) analysis (18) of one of the incubated plasmas revealed the  $^{14}\text{C}$  to be distributed as 96%  $^{14}\text{C}$ -DEHP and 4%  $^{14}\text{C}$ -MEHP; this pattern closely resembles that of unlabeled phthalates in human plasma stored in conventional PL-146 blood bags. Following bolus infusion of the  $^{14}\text{C}$ -DEHP-laden autologous plasma, serial plasma, urine and stool samples were obtained and counted for  $^{14}\text{C}$  activity.

As can be seen in Figure 1, plasma  $^{14}\text{C}$  concentration rapidly declined, so that by 90 min after infusion there was less than 5%, and after 12 hr there was less than 1% of the initial  $^{14}\text{C}$  concentration. Characterization of the chemical structures of the  $^{14}\text{C}$  compounds in plasma during the first 30 min after infusion, revealed rapid conversion of DEHP to MEHP and more fully oxidized MEHP derivatives (Fig. 2).

Figure 3 shows the buildup of  $^{14}\text{C}$  in urine following the infusion. It can be seen that by 4 hr after infusion, greater than 50% of injected  $^{14}\text{C}$  had

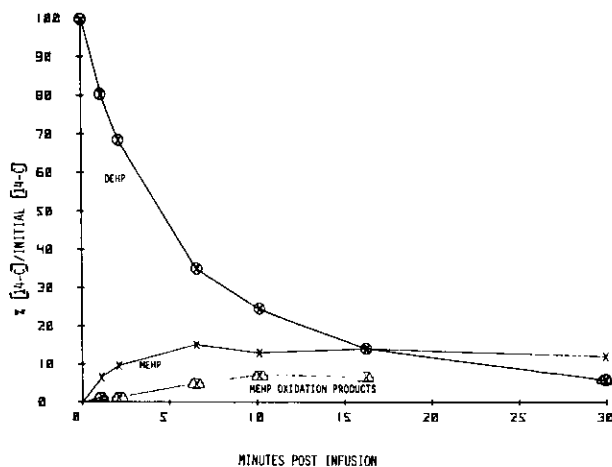


FIGURE 2. Plasma DEHP and metabolites of DEHP during the first 30 min following injection of  $^{14}\text{C}$ -DEHP leached into autologous African Green monkey plasma (13).

been excreted in the urine, and that by 24 hr, greater than 70% had been excreted by the kidneys.

Table 1 reveals the chemical structure of DEHP metabolites in urine of the African Green monkey as identified by GC-MS (18). The predominant metabolites were the 5-ethyl, isohexanol monoester of phthalic acid and MEHP. More than 80% of urinary metabolites were conjugated to glucuronide. The cumulative urinary excretion patterns of DEHP metabolites during the first 4 hr are plotted in Figure 4.

Fecal  $^{14}\text{C}$  excretion accounted for up to 8% of the injected  $^{14}\text{C}$  by 48 hr after infusion (Fig. 5). The theoretical cumulative urinary excretion of  $^{14}\text{C}$  at infinite time (beyond 4-5 days) coupled with the measured fecal excretion of  $^{14}\text{C}$  accounted for the entire injected dose of  $^{14}\text{C}$ -DEHP.

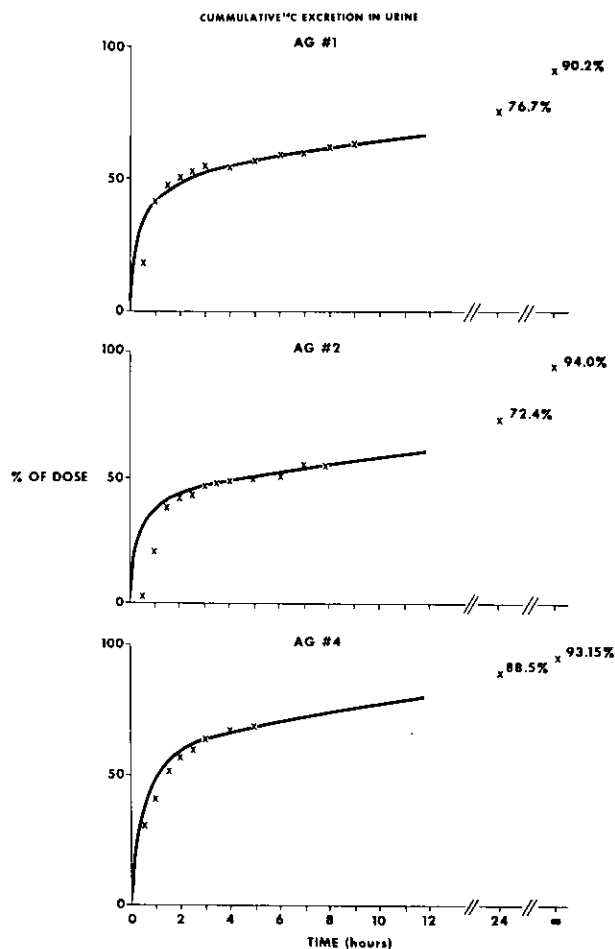


FIGURE 3. Cumulative  $^{14}\text{C}$  excretion in urine of three African Green monkeys following bolus injection of  $^{14}\text{C}$ -DEHP leached into autologous plasma. The solid line running through the data (x) was generated from a computer fit of the data (13).

Table 1. DEHP metabolites in urine of an African Green monkey (AG#4).

Metabolite		
Code	R <sup>a</sup>	% of dose 4 hr after infusion
12 B	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CHOH-CH <sub>3</sub>	26.1
MEHP	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	19.6
Unhydrolyzed <sup>b</sup>		5.4
11	CH <sub>2</sub> -CH(CH <sub>2</sub> CH <sub>2</sub> OH)-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	3.2
10	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CO-CH <sub>3</sub>	3.1
11 X	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CHOH-CH <sub>2</sub> -CH <sub>3</sub>	2.7
9 E	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -COOH	2.7
DEHP <sup>c</sup>	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub> <sup>c</sup>	<1.8
9 A	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -COOH	Trace
9 C	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -COOH	Trace
13 B	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>2</sub> OH	Trace
Phthalic acid	H	Trace
Total		66.9

<sup>a</sup>Structure: *o*-C<sub>6</sub>H<sub>4</sub> (COOX)COOR, where X = glucuronide or H.

<sup>b</sup>By  $\beta$ -glucuronidase or sulfatase.

<sup>c</sup>X = R.

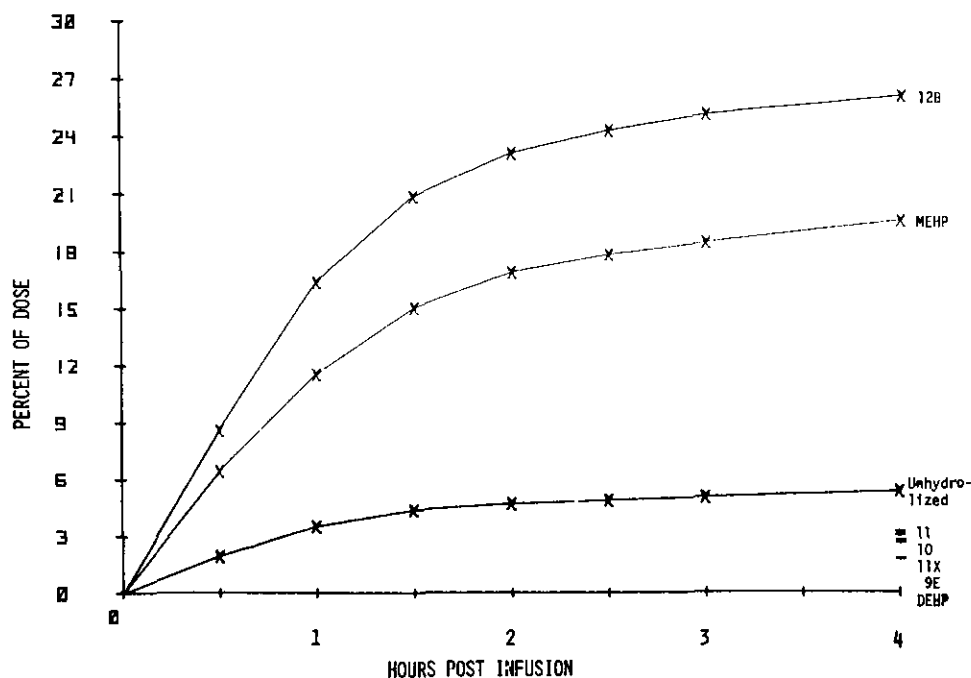


FIGURE 4. Cumulative excretion of individual DEHP metabolites in urine of one African Green monkey (AG 4) following bolus injection of <sup>14</sup>C-DEHP in autologous monkey plasma. The metabolite codes are the same as those in Table 1.

## DEHP Disposition and Metabolism in Man

A detailed study of the disposition and metabolism of DEHP in two cancer patients receiving larger doses of DEHP via platelet concentrate infusions has been reported (13, 19). One patient (case I) received 94.7 mg of DEHP in 4 hr, and

another (case II) received 174.3 mg of DEHP in 1.5 hr. Figure 6 shows the cumulative urinary excretion of all DEHP derivatives. More than 50% of the infused dose appeared as DEHP derivatives in urine within 6 hr. This pattern is similar to that seen in the African Green monkey (cf. Fig. 3).

Table 2 is a list of the chemical structures of the urinary metabolites (case II) which were identified by GC-MS (18). As in the African Green monkey,

Table 2. DEHP metabolites in human urine (case II).

Metabolite		
Code	R <sup>a</sup>	Cumulative % of dose 24 hr after infusion <sup>b</sup>
12-B	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CHOH-CH <sub>3</sub>	23.4
11	CH <sub>2</sub> -CH(CH <sub>2</sub> CH <sub>2</sub> OH)-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	8.3
10	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CO-CH <sub>3</sub>	7.7
MEHP	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	6.9
11-X	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-CH <sub>2</sub> -CHOH-CH <sub>2</sub> -CH <sub>3</sub>	6.1
9-E	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -COOH	4.7
9-D	CH <sub>2</sub> -CH(CH <sub>2</sub> COOH)-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	1.5
9-B	CH <sub>2</sub> -CH(COOH)-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub>	1.2
13-B	CH <sub>2</sub> -CH(C <sub>2</sub> H <sub>5</sub> )-(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>2</sub> OH	Trace
Total		59.8 <sup>c</sup>

<sup>a</sup>Structure: *o*-C<sub>6</sub>H<sub>4</sub>(COOX)(COOR), where X = glucuronide (80%) or H.

<sup>b</sup>Each is underestimated by < 2% due to sample loss.

<sup>c</sup>Underestimated by ≈ 6.1%.

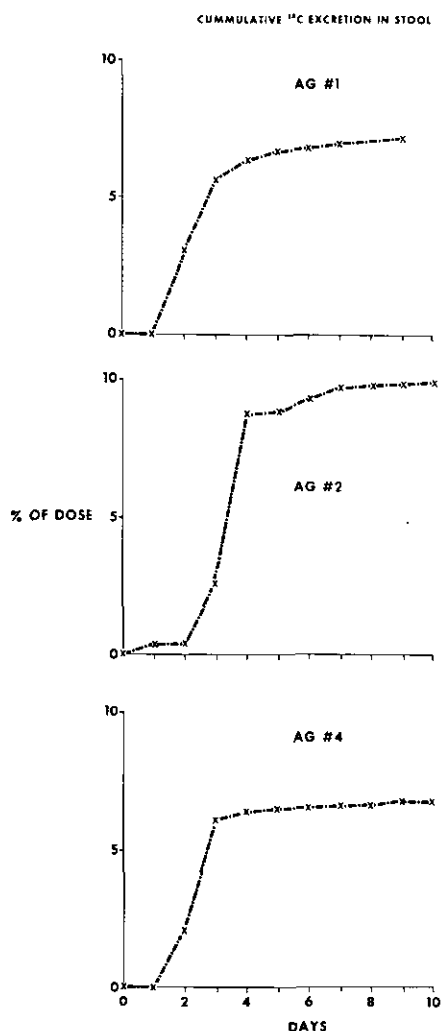


FIGURE 5. Cumulative excretion of <sup>14</sup>C in stool of three African Green monkeys (AG 1, 2, 4) following injection of <sup>14</sup>C-DEHP in autologous plasma.

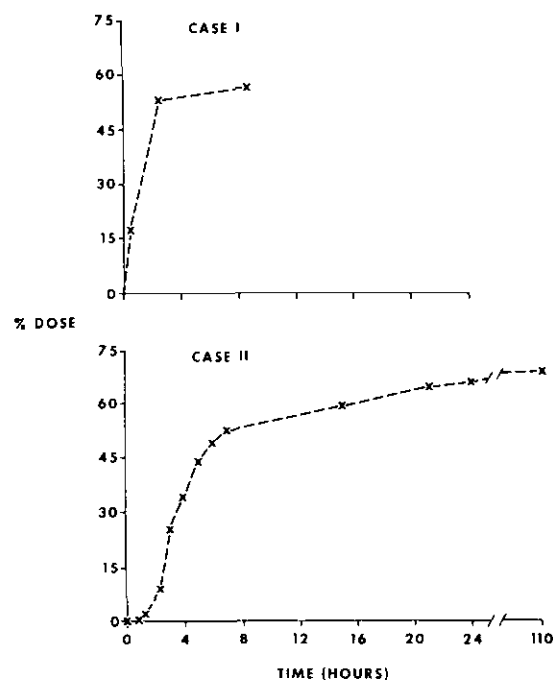


FIGURE 6. Cumulative urinary excretion of total DEHP metabolites in two cancer patients following platelet concentrate infusions containing leached DEHP.

the predominant metabolite was the 5-ethyl, isohexanol monoester of phthalic acid, but in contrast to the urinary metabolite pattern in the monkey, MEHP is fourth on the list and is followed by four more fully oxidized derivatives of MEHP. As in the monkey, approximately 80% of the urinary metabolites were conjugated to glucuronide.

Figure 7 shows the cumulative urinary concentrations of various DEHP derivatives in case II throughout the first 24 hr after infusion.

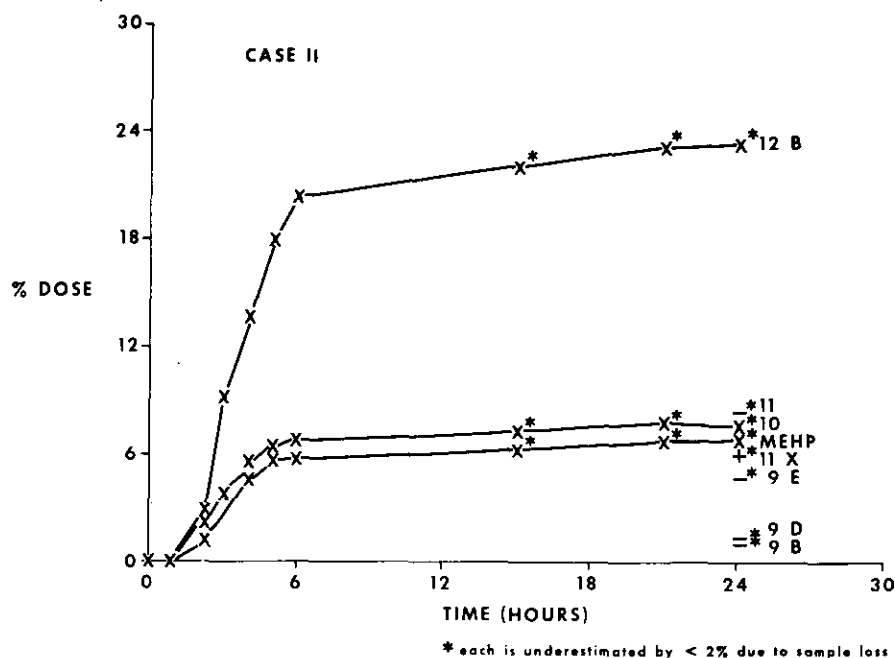


FIGURE 7. Cumulative urinary excretion of individual DEHP metabolites in a cancer patient (case II) following a 1.5-hr infusion of concentrated platelets containing 174.3 mg of leached DEHP. The metabolite codes are the same as those in Table 2. Asterisks (\*) denote values underestimated by < 2% due to sample loss.

A study of the disappearance of DEHP in human plasma following infusion of DEHP-laden platelet concentrates (20) enabled its pharmacokinetic characterization (21). Plasma disappearance half-life was  $30 \pm 12$  min, apparent distribution volume was  $2819 \pm 383$  ml/m<sup>2</sup> and clearance was  $78 \pm 20$  ml/min-m<sup>2</sup> ( $n = 6$ ). Based upon these average pharmacokinetic parameters, Figure 8 shows the plasma DEHP time profile for a simulated platelet infusion in which 90 mg DEHP in 530 ml of pooled platelet concentrate is infused in a 30-min period into a 70-kg man. The DEHP is initially distributed into a volume which is slightly larger than blood volume, rapidly distributes to other tissues and disappears from plasma so that it is essentially undetectable 4 hr after infusion.

## Discussion

Consideration of these kinetic and metabolic data in the light of potential toxicity in humans leads to some preliminary answers and invites new questions. The restricted apparent distribution space, rapid disappearance from plasma, rapid urinary excretion and extensive hydrolysis, oxidation, and glucuronide conjugation could all be interpreted as "detoxification" mechanisms. Such an interpretation, however, requires knowledge of tissue sensi-

tivities to DEHP and its metabolites. In the absence of such information, DEHP disposition data contribute to our assessment of potential DEHP toxicity in several ways. For instance, the extensive metabolism and rapid excretion of DEHP metabolites cast serious doubt on the contention that DEHP found in human tissues is a result of blood transfusions or exposure from other environmental sources; contamination during sample preparation and assay appears to be a more likely explanation. Moreover, the rapid excretion of the bulk of DEHP metabolites within 8 hr following infusion of blood products suggests that DEHP-related toxicity is more likely to be acute in onset rather than a late-appearing or long-term effect. Any toxicity observed may be the effect of a metabolic product of DEHP rather than the parent compound. In this regard, the reports of MEHP accumulation during blood product storage (14, 15) and description of its hepatotoxicity in rodents (17) should focus future research efforts on this compound in particular. Moreover, since the enzymes responsible for hydrolysis, oxidation and conjugation of DEHP and MEHP may be located primarily in the liver, this organ, as well as the kidney, will be exposed to relatively high concentrations of DEHP derivatives. This knowledge should focus DEHP toxicity research on these two organs, as well as alert investigators to

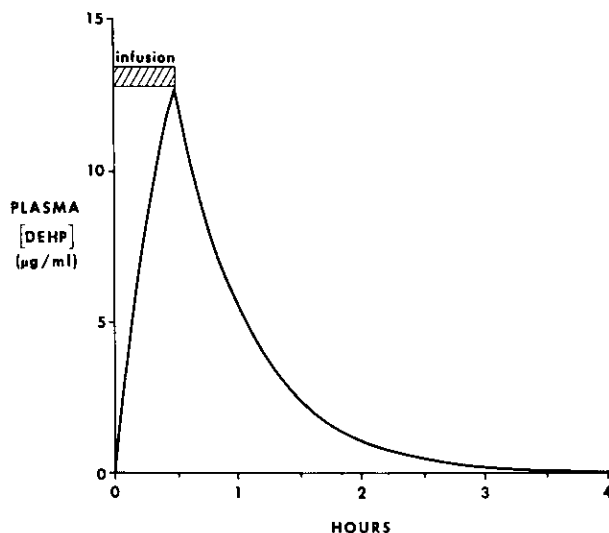


FIGURE 8. Simulated human plasma DEHP concentrations during and after a 30-min infusion of 530 ml of pooled 72-hr-old platelet concentrate (90 mg DEHP total). The simulation is based on average pharmacokinetic parameters derived (21) from data reported on six patients receiving DEHP-rich platelet concentrates (20), and assumes a body surface area of 1.73 m<sup>2</sup>.

the possibility that toxicity may appear with altered DEHP metabolism or excretion due to hepatic or renal disease.

There is no evidence at present that humans exposed to DEHP via DEHP-laden blood products or from other sources experience any adverse effects from DEHP exposure. The studies of DEHP disposition reviewed in this paper demonstrate that DEHP administered parenterally to primates and humans is rapidly converted to conjugated hydrolyzed oxidation products of MEHP which are rapidly excreted in the urine. These metabolic processes account for elimination of more than 50% of the infused DEHP by 8 hr after infusion, while elimination of the entire dose is virtually complete in 4-5 days. Whether these dispositional events account for the lack of observed human toxicity from DEHP remains an unanswered question.

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